

Chapter 3

Metal ions speciation in the environment: Distribution, toxicities and analyses

V.K. Gupta, Imran Ali and Hassan Y. Aboul-Enein

Abstract

Metal ions are the most toxic pollutants due to their acute toxicities, carcinogenicities and non-biodegradability. Speciation of metal ions is the most important area due to the different toxicities of the same metal ion species. This chapter describes the distribution of metal ions in water, sediment, soil, air, aquatic and terrestrial biota. Besides, discussion was also presented on metal ions toxicities and their analysis by chromatographic, capillary electrophoretic, spectroscopic and other methods. Some data of distribution and toxicities are also given in tabular form. A comparison of the activities of organic and inorganic metal ion species in biological systems is also included.

3.1. Introduction

Among various pollutants metal ions are very dangerous contaminants due to their acute toxicities, carcinogenicities and non-biodegradability. Transition metals, lanthanides and actinides exist in different oxidation states and form various species, e.g., arsenic (As) as As(III), As(V) and organic arsenic species (Caruso et al., 2000) and the concept is known as speciation. The different oxidation states of a particular metal ion possess different physico-chemical properties. Mainly, these oxidation states differ in their redox potential, complexation and hydration properties. It is very important to observe that these different oxidation states have different toxicities. The concept of the speciation dates back to 1954 when Goldberg (1954) introduced the term of speciation to improve the understanding of the bio-geochemical cycling of trace elements in seawater. Kinetic and thermodynamic information together with analytical data make it possible to differentiate between oxidized versus reduced, complexed or chelated versus free metal ions in dissolved form. The measurement of the total concentration of a metal ion cannot provide the

information about the actual physico-chemical properties required for understanding its toxicity, bio-transformation, etc. Thus, in order to obtain information on toxicity and bio-transformation of a metal ion in the environment, speciation is needed of the individual element. Because of these points the knowledge of metal ion speciation is essential for the environmental, industrial and clinician point of views. Therefore, the scientific community and other regulatory authorities are asking data on speciation rather than the total metal ion concentrations. In view of these points, attempts have been made to discuss the distribution, toxicities and analyses of metal ions in this chapter.

3.2. Distribution

Originally, the concentrations of elemental species of anthropogenic origin was zero but today they are found widely in the ecosystem as they have been continued to be distributed in a manner that affects the life cycle. Total trace element concentration may be static and the species may be highly dynamic. Therefore, metal ions change continuously with respect to the changes in the environmental parameters such as pH, concentration of ligands for complex formation, the physiological state of a cell and state of health of a living body. The thermodynamic and kinetic stability of elemental species in the environment has also to be taken into account. Unstable species in the environment are predominant and this state requires some special attention. Basically, all metal ions are present in the earth's crust in different abundance and oxidation states. There are different ores containing various metal ions and these find their ways from these ores to the different components of the ecosystem. Some of them get dissolved into aquifers and contaminate groundwater while the others are extracted by human beings and used for different useful purposes. During all these activities, metal ions find their ways to the surface water, air, vegetables, other foodstuffs, etc. and finally enter the human bodies. Metals occur in the nature mostly in the combined forms but, sometimes, in the free state too. The active metal ions are found in the form of their salts while metals, which have little or no affinity for oxygen and water, are found in the free state. The distribution of metal ions is discussed herein in the different components of the ecosystem.

3.2.1. Distribution in water

Water is the main component of the ecosystem and unfortunately it is the most contaminated by toxic metal ions. All forms of water resources, i.e.,

ground, surface and waste are contaminated due to heavy toxic metal ions. The oxidation of the different metal species causes metal ions to become soluble and enter into the surrounding environment through the drainage water. The knowledge of the geographic distribution of metal ions species in the natural water system is important for the environmental consideration of the geochemical and biological cycling of the element. Furthermore, this also provides insight into the geochemical process responsible for elevated metal ion concentrations in the different geological environment.

Nowadays, arsenic disaster contamination is the worldwide problem and more than 20 countries are suffering from this (Chatterjee et al., 1995; Das et al., 1995a). The poisoning of groundwater due to mercury, cadmium, lead and chromium has been reported in some parts of the world. Timerbaev et al. (1999) reviewed the speciation of metal ions in tap water (groundwater) and reported the presence of arsenic, strontium, cadmium, chromium, copper, iron, etc. The status and the trend of river water pollution due to toxic metal ions in India has been discussed and described by Prebha and Selvapathy (1997). The Greenland snow and ice cap has been shown to contain universal archives of the large-scale atmospheric pollution of the Northern Hemisphere by lead and other metal ions (Adams et al., 1998). The first evidence of global contamination by lead was reported by Murozumi et al. (1969), who detected lead in snow samples covering the period from 1753 to 1965. This discovery had suffered from controversy but later recognized by the assessment of natural lead levels in old Greenland ice dated 5500 years BC (Ng and Patterson, 1981). Pleßow and Heinrichs (2000) presented the speciation of various trace elements in acidic pore waters from waste rock dumps. Pansar-Kallio et al. (Pansar-Kallio and Manninen, 1996, 1997) reported the speciation of chromium metal ions in wastewater. Similarly, Arar and Pfaff (1991) described the presence of hexavalent chromium in industrial wastewater. Carro and Mejuto (2000) reviewed the presence of organo-mercury species in different kinds of waters. Briefly, all types of water resources have been contaminated due to the heavy toxic metal ions.

3.2.2. Distribution in the sediment

A dynamic equilibrium is established between the sediment and water. Many reports have been published on the presence of heavy metal ions in the sediment. Due to the transfer process between different environmental components and the natural ability of the sediments; to accumulate metal ions, their concentrations are generally higher than those present in adjacent water and air. The enrichment of metal ions in the sediments by

anthropogenic pollution is controlled by geological, mineralogical, hydrological and biological factors. Tack and Verloo (1995) reviewed the presence of metal ions on to the sediment and soil. Förstner and Wittmann (1983) described the presence of arsenic, selenium, zinc, lead, cadmium, chromium, copper, cobalt, nickel, mercury, silver, antimony, etc. metal ions on the sediment of lakes in North America and coastal areas of North America and British islands.

3.2.3. Distribution in the soil

The soils also get contaminated by the irrigation process by using the groundwater containing heavy metal ions. The contamination of soil also occurs through the use of fertilizers and pesticides containing metal ions. Besides, some metal ions present in the air also contaminate soil through rain. In addition to these, some man-made activities such as industrial and domestic discharges are also contributing toward the contamination of the soils. The humus layer of the soil plays a crucial role and this layer acts as a natural bio-geochemical barrier that suppresses the percolation of metal ions with seepage water, and thus, strongly accumulates the elements (Goldschmidt, 1937). Many metal ions have been reported on the soil at different parts of the world. Some reviews have been published on the distribution of metal ions on the soil (Das et al., 1995b). Matschullat (2000) reviewed the status of arsenic on the soil and the author reported a variable concentration of arsenic in different soils.

3.2.4. Distribution in the air

The atmospheric is also not untouched by the contamination of metal ions due to man-made and natural activities. Metal ions into atmosphere spreaded over a large area depending on the temperature, humidity, wind speed, etc. The determination of metal ions into ambient air, aerosol and dust is a subject of many environmental studies. Metal ions may absorb onto dust particles, fly ashes, etc. into the atmosphere. The determination of metal ions in aerosol is increasing continuously (Dabek-Zlotorzynska and Dlouhy, 1994, 1995a, 1995b). Matschullat (2000) reviewed the presence of arsenic in the atmosphere and the author reported 28,230–54,270 tons per year as arsenic flux released from different sources. Some toxic metal ions present in rain may be considered as the pollution into air as the rainwater dissolved these metal ions from atmosphere. Förstner and Wittmann (1983) reported aluminum, antimony, arsenic, cadmium, chromium, cobalt, copper, iron, lead, mercury, nickel, selenium, silver, vanadium, and zinc into the air.

3.2.5. *Distribution in aquatic biota*

Information of metal ions at high concentrations in animals and plants is an indication of the contamination of the environment. The role of heavy metals in aquatic organisms has been reported in the literature with emphasis on the toxicities of the individual metal ion (Doudoroff and Katz, 1953). Both essential (copper, zinc, iron, cobalt) and non-essential elements (arsenic, cadmium, lead, mercury) are found in the different aquatic organisms. Metal ions accumulation in aquatic organisms is controlled by biotic and abiotic factors. The biotic factors are physiological behavior, pattern of life cycle, seasonal variation in the organisms, species specific and individual variability, etc. while the abiotic factors include temperature and dissolved oxygen of water, hardness, pH, salinity, presence of several organic and inorganic compounds.

3.2.6. *Distribution in the terrestrial biota*

All the terrestrial animals and plants depend on water supply from the ground and surface waters and, hence, it is a common observation to find toxic metal ions in these organisms. The evolutionary studies of heavy metal tolerance have contributed for dealing with contaminated soils on several levels. Such types of studies have provided evidence for the toxic affects of heavy metal contamination on non-adapted genotypes. The evolutionary studies have also shown that the mechanism for metal tolerance is uptake, not exclusion, such that metal-tolerant genotypes are also metal accumulators. This last insight, in conjunction with physiological research on metal-tolerant plants, has led to a growing use of plants as a part of a clean-up technology for dealing with contaminated sites (Salt, 1995; Adler, 1996). Bradshaw and Trans (1991), in particular, have conducted extensive experiments on the evolutionary properties of plants growing in contaminated sites, such as mine spoils. Their findings, including growth of plants in contaminated sites, are genetically adapted to be tolerant of heavy metals. Metal-tolerant plants do not compete well in non-contaminated sites. This adaptation of plants to heavy metal contamination has been of particular interest because it is a character that appears to have evolved in part in response to human disturbance (Antonovics, 1971, 1975; Bradshaw and Trans, 1991). Dwivedi and Dey (2002) explored the possibility of translocation of heavy metals into humans and animals, the authors studied 28 commonly used medicinal plants and estimated their heavy metal content. Besides, some metal ions have been reported in the different kinds of food products. Basically, the type of metallic species and their concentrations in foodstuffs depend on the

origin of the food products. For example, many metallic species have been detected in the seafood samples and the food products grown on the heavily contaminated soil and water resources. Aluminum is a ubiquitous element found in every food product. The other sources of aluminum are corn, yellow cheese, salt, herbs, spices, tea and tap water. Larsen et al. (1993) determined various arsenicals in the seafoods. Liang et al. (2003) reported mercury species in seafood samples. Benramdane et al. (1999) reviewed the presence of arsenic species in different foodstuffs. The distribution of metal ion species in the different components of the ecosystem is summarized in Table 3.1.

3.3. Toxicities

As discussed above different metal ions have different toxicities and therefore, the discussion on this issue is very important and discussed herein briefly. The changes in the degree of oxidation of an element have an important affect on the degree of bioavailability and toxicity (Stoeppler, 1992). The toxicities of metal ion species depend on age and sex of human beings. The biological activities of organic and inorganic metal ion species in living systems are given in Table 3.2. In oxidized environment As, Sb and Se appear mostly as oxyanions (Cutter, 1992). The valency state of a metal ion controls the behavior of metal ion in the aqueous system. For example, the toxicity of As(III) and Sb(III) is higher than that of their pentavalent species (Berman, 1980; Gesamp, 1986). The toxicity of the water-soluble species of arsenic varies in the order of arsenite > arsenate > monomethylarsonate (MMA) > dimethylarsinate (DMA) (Stugeron et al., 1989). Metals occur in the natural waters in different physico-chemical forms. Among these, simple hydrated metal ions are considered to be the most toxic forms while strong complexes and species associated with colloidal particles are usually assumed to be non-toxic ones. Organometallic compounds are more toxic than their corresponding inorganic species with the exception of arsenic.

Illmer and Mutschlechner (2004) studied the affect of pH on the toxicity of aluminum metal ion on *Arthrobacter* species. The authors reported EC₁₀₀ indicating a complete inhibition of microbial growth with 185 μM aluminum for *Arthrobacter* 1 and 11 mM of aluminum for *Arthrobacter* 2. People drinking arsenic-contaminated water generally showed arsenical skin lesions, which are a late manifestation of arsenic toxicity. Long-term exposure of arsenic-contaminated water may lead to various diseases such as conjunctivitis, hyperkeratosis, hyperpigmentation, cardiovascular diseases, disturbance in the peripheral vascular and

Table 3.1. Distribution of metal ion species in the ecosystem

Metal ion species	Ecosystem component	Ref.
Water		
Cd, Cu, Pb & Zn	Vesdre river, Belgium	Houba et al. (1983)
Cd, Cu, Cr, Mn, Pb & Zn	Toyhira river, Japan	Sakai et al. (1986, 1993)
Cd, Cr, Cu, Pb & Ni	Pawtuxet river	Zhang and Huang (1993)
Fe, Mn, Cd, Pb, Ni & Zn	Ravi river, Pakistan	Mohammed and Hayat (1996)
Co, Cd & Zn	Kishon river, Israel	Barak and Kress (1997)
Cd, Co, Pb & Zn	Seine river, France	Alexandrine et al. (1998)
Cd, Cr, Cu, Fe, Ni, Pb & Zn	Yamuna river, India	Agarwal (1997)
Heavy metals	Rivers, USA & Europe	Förstner and Wittmann (1983)
Monobutyltin, dibutyltin and tributyltin	Sea water	Botana et al. (2002)
Co, Cu, Fe & Zn	Pond water	Regan et al. (1994)
Methyl-, ethyl- and methylethyl lead	Rain water	Zufiaurre et al. (1997)
Lead and other metal ions	Northern Hemisphere	Adams et al. (1998)
Lead	Snow samples	Murozumi et al. (1969)
Sediment		
Methylmercury	Sediment	Lai et al. (1998)
Butyltin(I) and butyltin(II)	Marine Sediment	Whang and Whang (1997)
Monto-, di- and tributyltin	Sediment Gulf of Gdansk, Baltic Sea	Flandysz et al. (2002)
Tributyltin and triphenyltin	Marine sediment	Feng and Narasaki (2002)
Trimethyltin, triethyltin and triphenyltin	Sediment	Yang, et al. (1995)
Monobutyltin, dibutyltin and tributyltin	Sediment	Ceulemans and Adams (1995)
Monobutyltin, dibutyltin and tributyltin	Sediment	Cai et al. (1993)
Soil		
Al species	Soil	Göttlein and Blasek (1996); Mitrovic and Milacic (2000)
As species	Soil	Prohaska et al. (1999); Yang et al. (1996)
Cu & Cr	Soil	Krajnc et al. (1995)
Hg	Soil	Hempel et al. (1995)
Me-Hg	Soil	Carro et al. (1998)
Pt	Soil	Michalke et al. (1997)
Organic lead, antimony, tin	Soil	Hirner et al. (2000)
Rare earth elements	Soil	Wang et al. (2001)
Air		
Al species	Air	Tripathi et al. (2002)
Mn, Sr & Cd	Aerosol	Dabek-Zlotorzynska et al. (1995b)
Zn & alkaline earth metal ion	Dust particles	Fung et al. (1998)

Table 3.1. (Continued)

Metal ion species	Ecosystem component	Ref.
Trimethylbutyllead, dimethylethylbutyllead, triethylbutyllead, methyldiethylbutyllead, dibutyldiethyllead and tetrabutyllead compounds	Dust	Nerin et al. (1995)
Alkali and alkaline earth metal ions	Atmosphere	Dabek-Zlotorzynska et al. (1995b); Fung et al. (1995)
Aquatic biota		
Cd, Cu, Mn, Pb & Zn	Diatoms, Monterey Bay, California	Knauer et al. (1973)
Mn	Phytoplankton	Morris (1971)
Cu, Pb & Ag	Fucus vesiculosus	Lande (1977); Stenner and Nickless (1974)
Cr, Cu, Fe & Mn	Algae	Martin and Broenkow (1975)
As species	Algae	Bowen (1979)
As species	Crustacea, mollusca, porifera, chinodermata & coelentrata	Francesconi et al. (1994)
Hg species	Euphausiids, copepods, ctenophores & coelenterates	Knauer and Martin (1973)
Hg species	Zooplanktons	Skei et al. (1976)
Toxic metal ions	Crustaceans	Stenner and Nickless (1975)
Hg	Asellus aquaticus	Hasselrot and Göthberg (1974)
Zn	Platichthyes flesus	Papadopoulou (1978)
Hg & Se	Makaira indica	MacKay (1975)
MeHgCl & HgCl ₂	Perca flavescens	Freitas et al. (1974)
Diethylmercury & ethylmethylmercury Specie	Dog fish	Logar et al. (2000)
Terrestrial biota		
Aluminum organic acid complexes	Plant sap	Bantan et al. (1999)
Fe species	Mugineic acid family plants	Weber, et al. (2002)
Se species	Terrestrial plants	Jung et al. (2002); Montes- Bayon et al. (2002); Zhang and Frankenberger (2001)
Cd	Pig	Crews et al. (1989)
Al & silicon	Human serum	Wrobel et al. (1995)
As species	Human serum & urine	He et al. (2000)
Alkyl lead	Human serum	Pons et al. (1998)
MeHg, EtHg, PhHg & Hg(II)	Human urine	Yin et al. (1998)
Organotin compounds	Human organs	Jiang et al. (2000)
Organo As, Ge, Se, Sn & Pb	Human urine	Kresimon et al. (2001)

Table 3.2. A comparison of the activities of organic and inorganic metal ion species in biological systems (Furst, 1987)

Biological action	Organic species	Inorganic species
Adsorption	Yes	Yes
Plasma protein binding	Albumin	Globulin
Target organs	Yes	Yes
Metabolized	Yes	Yes
Excretion (main route)	Bile	Urine
Induction	Enzyme (P-450)	Metallothiein
Oxidation states	Not important	Important
Biodegradation	Yes	No

nervous systems, cancer of the skin and gangrene, leucomelanosis, non-pitting swelling, hepatomegaly and splenomegaly (Kiping, 1977; Yamamura and Yamauchi, 1980; WHO, 1981; Pershagen, 1983). Arsenite is 20–100 times more toxic than arsenate in marine environment (Neff, 1997) and 60 times more toxic in human beings (Ferguson and Gavis, 1972).

Cadmium is supposed to be the most toxic metal ion with nausea and vomiting at 15 mg l^{-1} concentration. The kidneys are the critical target of cadmium contamination. The disorders associated with kidney are renal dysfunction, hypertension and anemia. The other side affects of cadmium are extreme restlessness and irritability, headache, chest pain, increased salivation, choking, abdominal pain, diarrhea, tenesmus, throat dryness, cough and pneumonitis. The exposure of cadmium chloride aerosol in rats has been reported of tumor formation (carcinogenesis). The different cadmium compounds found carcinogenic in nature are Cd, CdS, CdO, CdSO₄ and CdCl₂. WHO guideline for cadmium is 0.003 mg l^{-1} . Trivalent chromium [Cr(III)] is essential for animal and human health and growth; at trace levels, with a safe and relatively innocuous level of 0.20 mg day^{-1} whereas hexavalent chromium [Cr(VI)] is a potent carcinogen and extremely toxic to the animals and humans. But recently, Mulyani et al. (2004) reported the oxidation of Cr(III) to Cr(VI) leading to toxicity of trivalent chromium in the biological systems. The main notorious affects of Cr(VI) are on liver, kidney, respiratory organs with hemorrhagic problem, dermatitis and ulceration of the skin for chronic and subchronic exposure.

Lead is the most significant toxin of the heavy metals and affects are of toxicological and neurotoxic in nature, which include irreversible brain damage. Inorganic forms of lead typically affect the central nervous system (CNS), peripheral nervous system (PNS), hematopoietic, renal,

gastrointestinal, cardiovascular and reproductive systems. The various lead compounds reported to be carcinogens are PbO , PbCrO_4 , $\text{Pb}_3(\text{PO}_4)_2$ and PbAc_2 . Inorganic mercury compounds are toxic to kidney leading to neurological and renal disturbances while organic mercury compounds are very toxic to the CNS. Besides, discouragement, irritability, personality changes, learning disabilities, muscle tremors, jerky gait, spasms of extremities, inflammation of mouth and gums, swelling of salivary glands, excessive flow of saliva and loosening of teeth are the other hazardous affects of mercury poisoning. The mechanisms of toxicity and, particularly, the potential carcinogenicity of inorganic mercury are still under debate. The results of mutagenicity and genotoxicity testing with mercury have been inconsistent, i.e., mercury induces DNA single strand breaks at low concentrations in mammalian cells but has not proved mutagenic in several bacterial mutagenicity assays. Kungolos et al. (1989) studied the affects of six different forms of mercury on the growth of the yeast *Saccharomyces cerevisiae* using five kinds of strains of *S. cerevisiae*. In terms of EC_{50} toward the wild-type strain, the toxicity order for the inorganic forms was $\text{Hg}(\text{NO}_3)_2 > \text{HgSO}_4 > \text{HgCl}_2$. The different toxicities of various metal ion species are given in Table 3.3.

3.4. Analyses

Of course the analysis of the total concentration of metal ions is required and essential. Many reports are available in the literature on the analyses of metal ions but the data presented is not reliable. It is due to the different toxicities of various oxidation states of a particular metal ion, which makes the toxicity concept confused. The speciation of metal ions in the environment is a difficult job as the concentrations of heavy metals in the environment are generally very low. Moreover, sometimes, the physical and chemical properties of two or more species may be similar which makes the speciation again a challenging issue. Speciation strategy contains a careful plan or method or the art of developing or employing stratagems toward a goal. Scientists hope to learn everything about the elemental species, i.e., composition, mass, the bio- and environmental cycle, the stability of the species, its transformation and the interactions with inert or living matter. The work involved to achieve this goal is, however, challenging, if not impossible to complete. Therefore, a choice has to be made to identify the most important issues as elemental speciation studies are pursued. A first group of compounds to be studied very closely are those of anthropogenic in origin. In speciation studies, a lot of attention must to be paid to the stability. Species stability depends

Table 3.3. Toxicities of the different metallic species

Metallic species	Animals	LD ₅₀ (mg kg ⁻¹)
Arsenicals (Andraea, 1986; Kaise & Fukui, 1992; Penrose, 1974)		
Potassium arsenite	Rat	14
Calcium arsenate	Rat	20
MMA	Rat	700–1800
DMA	Rat	700–2600
Arsenobetaine	Rat	10,000
Lead compounds (Boyland et al., 1962; van Esch et al., 1962; Furst et al., 1976; Kanisawa and Schroeder, 1969; van Esch & Kroes, 1969)		
Pb-arsenite	Rat	100
Pb-naphthinate	Rat	5100
Pb-nitrate	Guinea pig	1300
Pb-chloride	Guinea pig	2000
Pb-fluoride	Guinea pig	4000
Pb-oleate	Guinea pig	8000
Mercury compounds (Cassidy & Furr, 1978; EPA, 1980)		
HgCl	Rat	210
HgI	Mouse	110
HgNO ₃	Mouse	388
HgO	Rat	8.0
MeHg	—	10.0
EtHg	—	40.0
Organotin species (Merian, 1991)		
Bis(tributyltin) oxide	Rat	150–234
Trimethyltin hydroxide	Rat	540
Triphenyltin hydroxide	Rat	125
Trimethyltin acetate	Rat	9.1
Triethyltin acetate	Rat	4.0
Tributyltin acetate	Rat	4000
Antimony compounds (Furst, 1987)		
Sb(III) or Sb(V)	Rat	100
Sb ₂ O ₃	Rat	3250
Sb ₂ S ₃	Rat	1000
Sb ₂ O ₅	Rat	4000
Sb ₂ S ₅	Rat	1599

on the matrix and on the physical parameters, such as temperature, humidity, UV light, organic matter, etc. The isolation and purification of the species, the study of the possible transformation through the procedure, their characteristics and interactions are also important. New analytical procedures have to be devised, including appropriate quantification and calibration methodologies.

Various advanced techniques have been developed to overcome this problem. The different analytical methods used for metal ion speciation include spectroscopic, polarographic, radiometric, chromatographic and electrochemical. Various technologies used for metal ion speciation are shown in Fig. 3.1. Among these techniques, chromatographic and electrophoretic methods have been used frequently due to their ease of operation and reproducible results. The spectroscopic methods of the speciation involve the use of various spectroscopic techniques such as UV visible, flame atomic absorption, atomic emission, hydride generation, graphite furnace, etc. (Krull, 1991; Jain and Ali, 2000). Besides the spectroscopic methods of speciation, polarography, voltammetry and radiometry have also been used for the speciation of metal ions (Pretty et al., 1990, 1992, 1993).

3.4.1. Chromatographic methods

The chromatographic methods involve the use of gas or liquid separately as the mobile phases. Therefore, the former kind of chromatography is called gas chromatography (GC) while the later is termed as liquid chromatography (LC). Due to some problems associated with gas

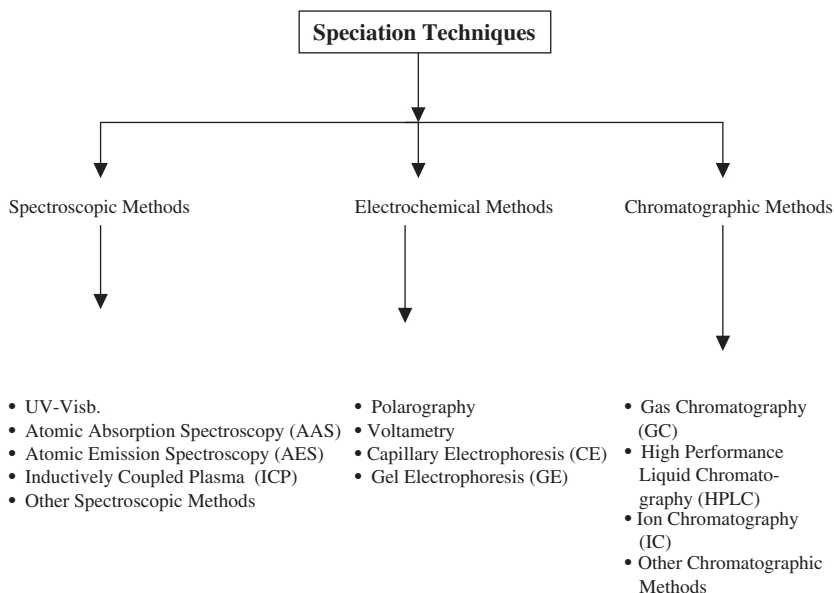


Figure 3.1. Different techniques used for meat ion speciation.

chromatography it could not be accepted as the method of the choice for the speciation of metal ions. The major disadvantage of GC is its requirement of the conversion of inorganic metal ions into organic and volatile metal ion derivatives, which is carried out by derivatization process. Contrarily, LC is the only and the best remaining technology for the speciation of a wide variety of metal ions. The main advantages of LC are its ability to speciate metallic species in the environmental samples directly. During the course of time various types of liquid chromatographic approaches were developed and used in this concern. Most important liquid chromatographic methods are high performance liquid chromatography (HPLC) and ion chromatography (IC). However, sub- and supercritical fluid chromatography (SFC), capillary electrochromatography (CEC) and thin layer chromatography (TLC) modalities can be used for this purpose.

Among the various liquid chromatographic techniques mentioned above, IC remains as the best modality due to its several advantages in comparison to others. The high speed, sensitivity and reproducible results make IC as the method of choice in the world. About 80% metal ion speciation has been carried out using IC mode of liquid chromatography. Various types of columns are available in IC, which make this technique popular in metal ion speciation. A variety of mobile phases including normal, reversed and new polar organic phases are used in IC. The composition of the mobile phases may be modified by the addition of various aqueous and non-aqueous solvents. Ion chromatography is a form of liquid chromatography that uses ion exchange resins to separate atomic or molecular ions based on their interaction with the resin. Its greatest utility is for analysis of ions for which there are no other rapid analytical methods. Most ion exchange separations are carried out with metallic pumps and columns. The column packing for ion chromatography consists of ion exchange resins bonded to inert polymeric particles. The optimization of metal ion speciation is carried out by a number of experimental parameters.

The use of supercritical fluids as the mobile phases for chromatographic separation was first reported more than 30 years ago, but most of the growth in SFC has occurred recently. In chromatographic systems, the solute diffusion coefficients are often of the order of higher magnitude in supercritical fluids than in tradition liquids. Contrarily, the viscosities are lower than those of liquid (Weast, 1973). At temperature below T_c and pressure above P_c , the fluid becomes a liquid. On the other hand, at temperature above T_c and pressure below P_c , the fluid behaves as a gas. Therefore, supercritical fluids can be used as a part of a mixture of liquid and gas (Berger, 1995). The commonly used supercritical fluids (SFCs)

are carbon dioxide, nitrous oxide and trifluoromethane (Weast, 1973; Schoenmakers, 1988; Berger, 1995). The compatibility with most detectors, low critical temperature and pressure, low toxicity and environmental burden and low costs make carbon dioxide as the supercritical fluid of choice. The main drawback of supercritical carbon dioxide as mobile phase is to be seen in its inability to elute more polar organic derivatives of metal ions and other compounds. This can be improved by the addition of organic modifiers to the relatively apolar carbon dioxide.

Basically, capillary electro-chromatography (CEC) is a hybrid technique, which works on the basic principles of capillary electrophoresis and chromatography (Cronin and Pizarello, 1997). This mode of chromatography is used either on packed or tubular capillaries/columns. The packed column in CEC was first introduced by Pretorius et al. (1974), while the open tubular CEC was presented by Tsuda et al. (1982). In 1984, Terabe et al. (1984) introduced another modification in liquid chromatography, i.e. micellar electrokinetic capillary chromatography (MECC). Of course this mode also depends on the working principles of capillary electrophoresis and chromatography but also involves the formation of micelles. The high speed, sensitivity, lower limit of detection and reproducible results make CEC and MECC as the methods of choice in the analytical science. However, these methods could not be used frequently for speciation as these techniques are not fully developed and the research is underway on this subject. Many research papers and reports have appeared in the literature on metal ion speciation using chromatographic techniques, all of which cannot be summarized in this chapter. However, interested readers should consult some review articles on this issue (Robarts et al., 1991; Cagniant, 1992; Quevauviller, 1996; Sarzanini and Mentasti, 1997, 1999; Nielen et al., 1998; Liu and Lee, 1999; Pereiro and Diaz, 2002; Ponce de Leon et al., 2002).

3.4.2. Capillary electrophoretic method

In the last decade, a newly developed technique called capillary electrophoresis (CE) has emerged as one of the tools for the speciation of metal ions. CE has certain advantages in comparison to the other techniques. The advantages of CE include inexpensive running cost, simple, high speed of analysis, greater separation efficiency, unique selectivity and high degree of matrix independence, all of which make it ideal for the speciation of metal ions (Gareil, 1990; Tessier and Turner, 1996; Baraj et al., 2000). Among the electrophoretic methods of metal ion speciation various forms of capillary electrophoresis such as capillary zone electrophoresis (CZE), capillary isotachopheresis (CIF), capillary gel

electrophoresis (CGE), capillary isoelectric focusing (CIEF), affinity capillary electrophoresis (ACE) and separation on microchips have been used. However, in contrast to others, CZE model was used frequently for this purpose (Chankvetadze, 1997). More than 100 research papers have been found in the literature on metal ion speciation using capillary electrophoresis but for quick search and information interested readers should consult some review articles on this subject (Dabek-Zlotorzynska et al., 1998; Liu and Lee, 1999; Timerbaev, 2002).

3.4.3. Spectroscopic and other methods

Spectroscopic methods of the speciation involve the use of UV-visible, flame atomic absorption, atomic emission, hydride generation, graphite furnace, etc. Some of them require the conversion of one form into another. Few metallic species form different colours with certain ligands and they can be speciated by UV-visible spectroscopy. For example, arsenic can be speciated by molybdenum blue and silver diethyldithiocarbamate methods. Even methods such as atomic emission and atomic absorption spectrometry coupled with flow injection analyser system (FIAS) give results that are more precise and exact when only one form of an element is present. Atomic absorption spectrometry with hydride generation is one of the most widely used methods for the speciation of some metal ions. For example, the speciation of arsenic is carried out by determining As(III) and the total arsenic, which is determined by the reduction of As(V) to As(III). The prior oxidation of arsenite to arsenate by KI has been reported (Bogdanova, 1984; Matsubara et al., 1987; Nasu and Kan, 1988; Tamari et al., 1989; Palanivelu et al., 1992). Besides, recently a method was developed for the determination of trace amounts of As(III) and total arsenic with L-cysteine as pre-reductant using flow injection hydride generation coupled with an in-house made non-dispersive AAS device (Yin et al., 1996). Inductively coupled plasma spectroscopy (ICP) involves the use of plasma formed at high temperature. This technique can be used for speciation after hyphenation with hydride generation unit. Lower limit of detection in ICP can be achieved by coupling it with mass spectrometer. Among electrochemical methods, potentiometry, polarography, cyclic, pulse and stripping voltammetric techniques are used for metal ion determination (Rajeshwar and Ibanez, 1997). The direct current polarography is able to detect different oxidation states of many metal ions. In addition to above, some reports are available on metal ion speciation by other techniques such as X-ray, X-ray fluorescence (XRF), EX-AFS, XA-NES spectroscopy, isotopic dilution, sensors, etc. Radiometric methods have been used for

metal radioactive ion species. However, this method can be applied for speciation of non-radioactive metal ions by producing artificial radioactivity. Some reviews have been published on metal ion speciation using these techniques and readers should go through these articles (Florence, 1986; Luque de Castro, 1986; Cornelis, 1992; Taylor and van Staden, 1994; Williams and D'Silva, 1994; Rosenberg, 2003).

3.5. Conclusion

About 75,000 chemicals are being in use everyday having different species and some of them are in direct or indirect contact with human beings and, hence, speciation is an integral part of the specification criteria, because only certain defined chemical species are permitted as sources of the essential elements. In the lack of legislation governing the speciation concept people are consuming toxic metal ion species, which result into some serious side affects and ailments. The lack of awareness about the speciation may be a serious and challenging problem to the world community in the near future due to the continuous contamination of our environment. A little attention has been paid for regulating metal ion speciation at International level. In view of these facts, it is very important to determine the toxicities of all metallic species in order to facilitate the speciation regulators. The toxicological data should be capable to recognize the social awareness. The analytical techniques should be developed in such a way, which can speciate metal ions into the real-world sample with high selectivity, sensitivity and reproducibility. A social awareness of science is required recognizing the speciation issue among the communities. Briefly, there is a great need for a think of risk, safety and communication associated with metal ion speciation at International levels. The politician and the regulatory authorities should come forward to stipulate the regulations and legislation to control the speciation of metal ions into our environment.

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